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Institutionen för husdjurens  
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Examensarbete 331  
45 hp E-nivå

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Uppsala 2011



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**Nyckelord:** Slaughter chickens, feed conversion, feces, absorption, small intestine, mucosa, villi, crypts, histology

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## Abstract

The objective of this study was to evaluate the influence of dietary glutamine (Gln) and glutamate (Glu) supplementation at the levels 0.5 or 1% in the diet of male broiler chickens (ROSS 308) on performance, intestinal morphology, number of *Clostridium perfringens* in cecum, faecal dry matter content and pH. Two hundred day old broilers were randomly allotted to 25 treatment groups (eight chickens per pen). Five experimental diets (0% Gln/Glu, 0.5% Gln, 1% Gln, 0.5% Glu and 1% Glu) were fed between 1 and 35 days of age. Treatments did not significantly affect broiler overall performance, intestinal morphology, length of the different small intestine segments, weight of spleen and liver in relation to body weight, number of *Clostridium perfringens* in cecum and faecal dry matter.

## Introduction

The market share of broiler chicken meat has increased in recent years due to the high demand for chicken meat. Therefore, it is desirable to improve the production and management systems so that the broiler industry could meet the market demand. This can be achieved by genetic selection, improved nutritional and hygienic conditions of feedstuffs and herds. One of the results from optimizing broiler production is that the feed intake per unit of body weight gain (feed conversion ratio; FCR) has been decreased. As a result, broilers may reach the target weight in fewer days. However, owing to the reduced FCR and shorter production periods, increased demands on feed quality have arisen. For a long period of time it has been cost benefit to supplement broiler diets with pure (synthetic) amino acids. (Dozier *et al.*, 2008). Feeding low protein diets results in impaired performances of broilers (Berres *et al.*, 2010). But, when low protein diets are supplemented with certain amino acids, broiler performance may be recovered (Berres *et al.*, 2010; Corzo *et al.*, 2005).

Amino acids are known as organic compounds comprising both an amino and an acid group (Wu, 2009). Deficient supply of amino acids could reduce growth and development, and lead to severe diseases. Besides the role of amino acids as proteins and peptides constituents, some amino acids (e.g. glutamine, arginine, leucine, proline, cysteine and tryptophan) are also involved in regulation of metabolic pathways, thereby affecting growth, maintenance, immunity, protein accumulation and health (Wu, 2009).

Functionally gastrointestinal tract (GIT) of a bird is responsible for the storage of food content, enzyme secretion and nutrients uptake. Small intestine of a newly hatched chicken comprises about 1.2- 2.6% of whole body weight (Murakami *et al.*, 2007). Interestingly, the GIT in birds produced for meat production such as broiler is longer and heavier than in layers (Yamauchi, 2002).

The small intestine is the main site of feed digestion, nutrients absorption and secretion of digestive fluids. The small intestine comprises three different segments duodenum, jejunum and ileum, they are normally investigated together due to the many

common characteristics (Junqueira *et al.*, 1992; Yamauchi, 2002). The small intestinal membrane consists of mucosa and sub-mucosa and which is recognizable with naked eye. Under magnification, intestinal villi and crypts are seen. The intestinal villi in duodenum are leaf shaped and changed steadily to finger shape as the ileum is reached (Junqueira *et al.*, 1992). In general, intestinal villi are 0.5-1.5 mm long (Junqueira *et al.*, 1992), and they are projecting out of the lamina and epithelium into the lumen of the small intestine (Yamauchi, 2002) (figure 1). The tall column shaped epithelial cells consist of absorptive, goblet, paneth cells and enteroendocrine cells laid on the surface of villi (Junqueira *et al.*, 1992; Yamauchi, 2002). The base of the intestinal villi is covered by young epithelial cells which are known as intestinal crypts (Yamauchi, 2002). All epithelial cells are continuously created at the base of the crypts in the stem cell zone, and then move to villi surface up to the tip (Sklan, 2004).

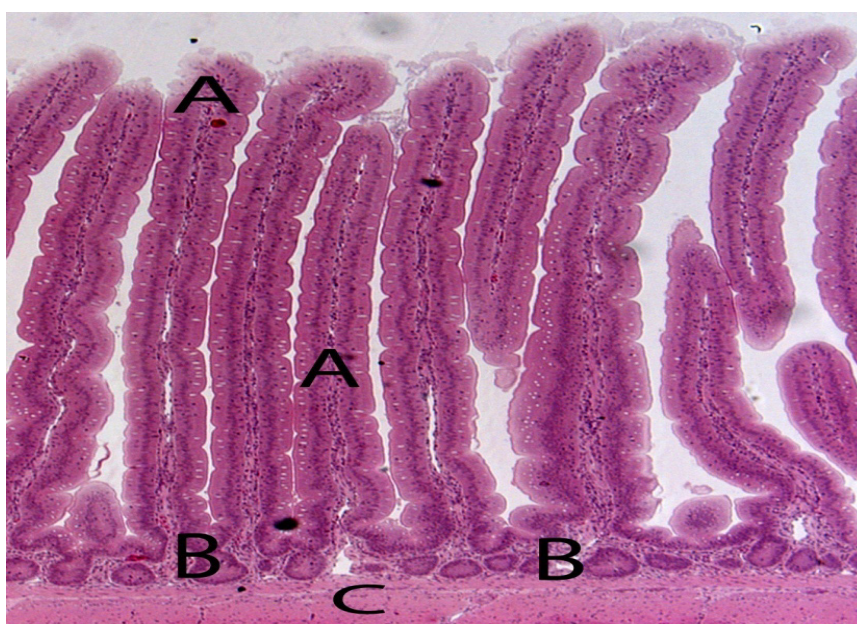


Figure. 1. Photomicrograph of the intestinal jejunum of 34 day old broiler. A, is indicating jejunal villi. B is showing blind ends of the villus where called crypts. C is showing smooth muscle layer.

Li *et al.* (2007) and Beisel (1996) reported that inadequate nutrition and infection are two major barriers to performance and survival of human and animals. Supplying diets with high-quality protein could improve resistance against disease. It has also been shown that using anti-microbial compounds in animal diets could improve both performance and health. For example, using antibiotics in poultry production results in better birds' performance via the effects on microbes in the gastrointestinal tract, particularly, in the first 9 days post hatch when the IgA concentration in small intestine is low (Mateos *et al.*, 2002). However, The European Union has banned the use of antibiotics as a growth promoter in animal diets due to the post-negative effects on consumer health (Mateos *et al.*, 2002), and Fasina *et al.* (2010) reported that there has been a rapid increase in the number of antibiotic resistant *Salmonella*. Hence, amino acids and probiotics have become alternatives to growth

promoters (e.g. antibiotics), to improve structure and health status of gastrointestinal tract (Mateos *et al.*, 2002).

Nitrogen emission is one of the main issues today in animal production (Deschepper and Groote, 1995). For instance Aletor *et al.* (2000) reported that in pigs, 65% of the absorbed nitrogen from the diet is excreted through faeces and urine. Poultry production also has this problem with nitrogen (N) emission because chickens are not able to retain all dietary protein (Deschepper and Groote, 1995). This has revived interest for lowering the crude protein in diets to reduce ammonia emission and N excretion in poultry production (Hussein *et al.*, 2001). Optimization of dietary amino acids composition by supplementing low protein diets with synthetic amino acids for both maintenance and meat production could improve efficiency of nitrogen and result in reduction of nitrogen emission (Deschepper and Groote, 1995; Ferguson *et al.*, 1997). For instance, Ferguson *et al.* (1997) reported that by balancing with synthetic amino acids they could reduce dietary crude protein fed to chickens by 2.5% and the N concentration in broiler manure was reduced hereby up to 21%.

Glutamate and glutamine are closely related non-essential amino acids naturally present in common feedstuffs. Glutamine is an amino acid of interest to human medical use (Newsholm *et al.*, 2003a). Hence, the potential use of glutamine in broilers diet has been discussed, and many benefits have been noted in different studies (Murakami *et al.*, 2007; Yi *et al.*, 2005; Bartell and Batal, 2007; Sakamoto *et al.*, 2006). However, in most of the studies the effects of glutamine have been investigated under adverse challenge conditions e.g. stress, inflammation and diseases (Yi *et al.*, 2005; Dai *et al.*, 2009; Soltan, 2009; Fischer da Silva *et al.*, 2007).

According to Wu, (2009); Tapiero *et al.* (2002) and Newsholm *et al.* (2003)a glutamine and glutamate may be converted to each other in different organs such as intestine, liver and kidney and both are related to the development of gastrointestinal tract of broiler chickens. Most attention has here been paid to glutamine, which is arterially distributed to the small intestinal mucosa. However, Reeds *et al.* (2000) proposed that dietary glutamate is the most important substrate.

To this date no experiments have been conducted on the use of pure glutamate supplementation in broiler diet. Therefore, the objective of this study was to investigate the impact of two non-essential amino acids glutamine and glutamate on growth performance and development of gastrointestinal tract of broiler chicken.

## **Literature review**

### **Glutamine and glutamate metabolism**

Glutamine (Gln) is a non-essential amino acid which is quantitatively the most abundant free amino acid in blood plasma compared to other free amino acids (Tapiero *et al.*, 2002; Newsholme *et al.*, 2003a; Murakami *et al.*, 2007; Bartell and Batal, 2007). Gln is

important for different physiological functions and maintenance of cell functions (Newsholme *et al.*, 2003a; Tapiero *et al.*, 2002). It acts as the substrate for several amidotransferases involved in the synthesis of purines, glucosamine, pyrimidines and asparagines (Watford, 2008; Li *et al.*, 2007). Gln is also involved in protein, peptide, and nucleic acid synthesis. It is available as source of oxidative energy and in the biosynthesis of glucose, amino sugars, and glutathione (Tapiero *et al.*, 2002; Newsholme *et al.*, 2003a). Glutathione is a tri-peptide which acts as an antioxidant cell protection against oxidative stress caused by e.g. peroxides (Cawthon *et al.*, 1999; Wang *et al.*, 1997). The end products of Gln catabolism could be either carbon dioxide or glucose (Watford, 2008; Herbert *et al.*, 1975). However, in most cells, Gln metabolism results in the production of L-glutamate and ammonia by the action of glutaminase (Newsholme *et al.*, 2003a; Tapiero *et al.*, 2002; Watford, 2008) (Figure 2). On the other hand, Gln can be produced by the combination of an amino group and glutamate (Glu) by the action of Gln synthetase. Glu derived from Gln, could be then further metabolized and used in the synthesis of glutathione, ornithine, arginine and proline (Watford, 2008; Herbert *et al.*, 1975). It appears that Glu may be involved in synthesis of new amino acids through donation of amino groups (Calder and Yaqoob, 1999) (Figure 3). Gln is involved in ammonia exchange between numerous tissues, and Gln acts as a precursor for ammoniogenesis in the gut and kidney (Tapiero *et al.*, 2002). Gln thus plays a role in the regulation of ammonia level in the body. In birds, ammonia is excreted in feces in the form of uric acid and Gln and Glu are involved in uric acid synthesis (Soltan, 2009; McDonald *et al.*, 2002).

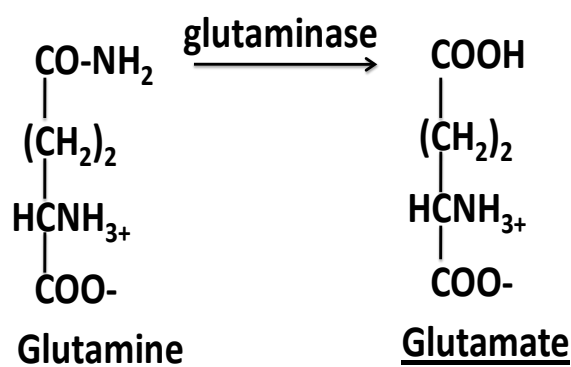


Figure 2. Chemical composition of glutamine and glutamate. Glutamine transforms to the glutamate at presence of glutaminase (Tapiero *et al.*, 2002).

The major site of dietary Gln absorption is small intestine (Souba, 1993; Tapiero *et al.*, 2002). However, according to Souba *et al.* (1990) in sepsis (potential severe inflammatory condition or blood poisoning) the absorption of Gln may increase by up to 10 folds. Skeletal muscles and lungs could be the major sites for Gln excretion (Tapiero *et al.*, 2002).





been shown that, luminal Glu is the major precursor of glutathione synthesis in the small intestine (Wu, 1998; Vernon and Ajami, 2000).

It has been found that dietary Gln may be the most important fuel for small intestinal mucosa (Wu, 1998) connected with the formation of Glu (Newholms *et al.*, 2003a; Reeds *et al.* 2000). Different studies have shown that addition of Gln to the diet of broilers could increase the relative weights of duodenum and jejunum (Bartell and Batal, 2007; Soltan, 2009). Newsholm *et al.* (2003a) and Li *et al.* (2007) pointed out that dietary Gln is known as an important constituent for maintenance of the gut integrity. For example, Yi *et al.* (2005) observed that addition of Gln into diet of broilers vaccinated with *Eimeria maxima* could reduce intestinal lesion score and promote intestinal development and increase maintenance of the intestinal integrity. According to Newholms *et al.* (2003a) and Reeds *et al.* (2000) there appear to be an important difference in the utilization of Gln and Glu for lumen replication in the small intestine, Gln being absorbed and utilized arterially in connection with formation of Glu, whereas Glu is absorbed and utilized directly in the lumen. This raises the questions whether dietary glutamate is an essential factor for the maintenance of mucosal health.

It is believed that longer villi in the small intestine could increase the food utilization efficiency in early stages of chicken life and result in better performances of broilers. Several studies have shown that Gln supplementation to the diet could increase the villi length in different segments of the small intestine. Soltan (2009) observed that broilers fed diet containing Gln had significantly longer villi in duodenum and jejunum compared to the control group. Interestingly, Murakami *et al.* (2007) showed that on day 14 post-hatch, broilers fed a diet supplemented with 10 mg vitamin E (vit E) along with 1% Gln (vit E  $\times$  Gln) had longer villi and deeper crypts in duodenum and deeper crypts in jejunum than broilers fed a diet containing vit E without Gln (vit E  $\times$  Gln free), or a diet supplemented with Gln without vit E (Gln  $\times$  vit E free). This may indicate that the interaction between Gln and vit E in the small intestine of broilers could be beneficial. In contrast, Wu *et al.* (1996) observed that an addition of 1% Gln had no effect on villi length in duodenum of 7 day old post-weaning pigs.

### **Glutamate and glutamine and the function of immune system**

Glu plays several roles in the metabolism and function of leucocytes (Li *et al.*, 2007). Immune system cells such as lymphocytes may utilize Gln as an energy source (Prabhu and Kalhan, 2007). Glu regulates inducible nitric oxide synthase (iNOS) in specific tissues such as brain (Li *et al.*, 2007). Expression of iNOS is considered as an essential mechanism in the protection against parasites, bacteria, fungi, malignant cells, intracellular protozoa and viruses in different animal species including both mammals and birds (Li *et al.*, 2007). Glu is involved in  $\gamma$ -aminobutyrate synthesis found in lymphocytes and macrophages. Lin *et al.* (1999) observed that immune-suppression in rats induced by chemotherapy was recovered by 83 % and 133 % after feeding diets containing 4 % and 8 % Glu respectively. Furthermore, Glu could act as an immediate substance in glutathione synthesis (Li *et al.*, 2007; Wu *et al.*, 2004). Glutathione is an important compound in elimination of oxidants and in modulating immune response (Li *et al.*, 2007; Wu *et al.*, 2004). Gln is also an essential substrate for development of lymphocytes and macrophages. Moreover, activity of immune

cells (e.g. T-cell proliferation, cytokine production, B-lymphocyte differentiation, antigen presentation and macrophage phagocytes), is increased in the presence of Gln. *In vivo* studies have shown that supplementation of Gln improves the function of the immune system. Soltan (2009) demonstrated that broilers fed a diet which contained 1% Gln had significantly higher counts of red blood cells, white blood cells and percentage of hemoglobin in comparison with broilers fed a diet without Gln. Calder and Yaqoob, (1999) reported that glutaminase (enzyme involved in glutamine deamination) activity increased in all lymphoid organs (e.g. spleen, thymus and lymph nodes) in response to pathogens. Barter and Batal, (2007) observed an increase in the relative weight of thymus and spleen of broilers fed diet containing 1% Gln in comparison with the group fed a control diet. Similarly, Sakamoto *et al.* (2006) showed that in 7 days old broilers fed a diet containing 1% Gln, only the relative weight of the spleen was increased but that other lymphoid organs had the same relative weight among the treatments. Further, Bartel and Batal (2007) observed that broilers fed Gln had higher IgA concentration level in serum, bile and intestine. IgA acts as a barrier against bacterial bonding to mucosal cells. Therefore, inclusion of Gln in the diet could induce the IgA production, increasing the immunity against bacteria and parasitic antigens.

## **Materials and methods**

### **Experimental design**

Two hundred day-old male broiler chickens from the female parent line of a commercial strain (Ross 308) were obtained from Swechick hatchery in Sweden and transferred to the Swedish University of Agriculture Science Research Center Funbo-Lövsta, Uppsala. Chickens were weighed and allotted into 5 groups randomly. Each treatment comprised 5 replicate pens (25 pens in total) with 8 birds in each. The pen size was 70x148 cm.

All chickens had *ad lib* access to water and feed and the diets were available as mash and pellets in first 7 days, and only as pellet from day 7 till last day. Diets were based on wheat and soybean meal and formulated to meet the chicken's recommended levels of nutrients. Diets contents were of similar nutritive value (Table1). The chickens were fed the same diet throughout the whole experiment.

Treatments contained supplements of glutamine and glutamate at two different levels (0.5–1%) for each one. Birds in treatments 1 and 2 were fed diets supplemented 0.5-1% Gln, respectively, on the expense of soybean meal, treatments 3 and 4 were fed 0.5-1% Glutamate Glu, respectively, on the expense of soybean meal. Treatment 5 birds were fed a control diet. The level of the glutamine and glutamate in the basal diet were not measured by chemical analysis but the content of glutamic acid was calculated using the Evonik/Degussa AminoDat® 3.3 (2006) information.

The chickens were maintained at 18-h light per day. Temperature was 32°C at chicken arrival to day five and was reduced half a degree per day in the following days. The trial

lasted 35 days. All the pens were equipped with nipple drinkers and a feeder and littered with wood shavings. The chickens were weighed every 7 days and food consumption was calculated every 7 days. The chickens were observed two times per day (every morning and evening) and then mortalities were recorded.

The experiment was conducted with permission of the Uppsala local ethics committee and in agreement with Swedish animal welfare regulations.

Table 1. Diets composition, calculated and analysed content of nutrients.

Ingredients, %	1% Gln	0.5% Gln	0.5%Glu	1% Glu	Control
Wheat	68	68	68	68	68
Soybean meal	24	24.5	24.5	24	25
Soya oil	2.5	2.5	2.5	2.5	2.5
Calciumcarbonate	1.6	1.6	1.6	1.6	1.6
Monocalciumphosphate	1.5	1.5	1.5	1.5	1.5
Salt	0.35	0.35	0.35	0.35	0.35
DL-Methionine	0.2	0.2	0.2	0.2	0.2
L-Lysine HCl	0.4	0.4	0.4	0.4	0.4
L-Threonine	0.25	0.25	0.25	0.25	0.25
Vitamins / premix	0.25	0.25	0.25	0.25	0.25
Glutamine	1	0.5	---	---	---
Glutamate	---	---	0.5	1	---
<i>Calculated composition</i>					
CP, g/kg	189.11	191.31	191.31	189.11	193.51
Lysine, g/kg	11.7	11.83	11.83	11.7	11.97
Met+ Cys, g/kg	7.95	8.01	8.01	7.95	8.08
Threonine, g/kg	8.12	8.21	8.25	8.12	8.29
Glutamic acid, g/kg	43	43	48	53	43
Na, g/kg	1.75	1.75	1.75	1.75	1.75
<i>Chemical Analysis</i>					
Dry matter (DM) %	88.6	87.7	88.6	89.1	87.7
Ash, g/kg DM	60	60	57	59	60
Crude Fiber, g/kg DM	32	31	33	35	32
CP, g/kg DM	232	228	228	226	220
Fat, g/kg DM	48	46	47	47	48
Ca, g/kg DM	10.1	10.2	9.7	10.4	10.4
P, g/kg DM	7.2	7.0	7.5	7.4	7.8
Mg g/kg DM	2.0	2.0	2.1	2.1	2.1
K g/kg DM	7.8	7.4	7.7	8.0	7.6
S g/kg DM	2.3	2.1	2.2	2.3	2.2

## **Faecal dry matter and pH**

Dry matter (DM) and pH of faeces were determined on day 7, 14, 21 and 28. Faeces from each pen were collected by using a plastic sheet after removing the littered floor of each pen and placing the chickens on a wire floor. After the faeces collection which took 6 hours, litter floors were reinstalled in the pens. Faecal samples were cleaned from wood shavings and stored in - 20 °C over night in separate nylon bags. The next day bags were left in room temperature to thaw and the content was carefully mixed. Approx 2 g of each sample was dried at 105°C over night. About 10 g of each faecal sample was put into a falcon tube and mixed with distilled water and then centrifuged in 3000 rpm (JOUAN G4-11) for 5 minutes until the solids had precipitated. Then pH was determined on the supernatant using a pH meter (Metrohm, 654 pH-meter).

## **Feed conversion ratio**

Food conversion ratio (FCR) was calculated for the whole period of production which is expressed by the following equation:

$FCR = \text{Food consumption (F)} / (W_2 - W_1)$ , in which  $W_2$  is live weight of the chickens in day 35 and  $W_1$  is live weight at day 0, including weight of dead birds.

## **Dissection and histology**

At 34 d of age one bird from each pen was randomly selected and weighed. Birds were stunned by a blow to the head and killed by cervical dislocation. All the birds were dissected immediately and 5 cm of mid-jejunum (15 cm proximal to Meckel's diverticulum) was rapidly excised. The segments were cut open along the length, pinned to small cork pieces and fixed in 2.5% phosphate buffered glutaraldehyde at 4 °C for 72 h.

At the same time the intestine, liver and spleen were collected. Then the intestine was divided in three sections: duodenum, jejunum and ileum (apex from ileocecal junction to Meckel's diverticulum). The length of the different sections was measured individually. One caecum was weighed and other one was transferred to a Petri dish, stored in an ice box and analyzed for the presence of *Clostridium perfringens* within 24 hours according to Waldenstedt *et al*, (2001). Liver and spleen were weighed separately.

For the histological evaluation fixed tissues were rinsed in 1/15 M phosphatebuffer and 2 slices were cut from each intestinal sample, (2 mm thick). The samples were embedded in the water soluble resin (Leica Histo-resin, Heidelberg, Germany). One slice of each sample was selected and cut on a microtome (Leica RM 2165, Leica Instruments, Germany) using glass knives (2 µm thickness). The sections were stained with Haematoxyline/Eosin and covered with Agar 100. The histochemical procedure was described in details by Ridderstråle (1991).

## **Image analysis and morphological evaluation**

In the morphometric study, all sections were coded. Images were captured using computer-aided light microscope (Nikon Microphoto-FXA, Bergström Instrument AB, Stockholm, Sweden). The magnification was 4X. The microscope was equipped with image analysis software (Easy Image Measurements 2000 & 3000, Bergström Instrument AB,

Stockholm, Sweden). The height of 5 villi and depth of 5 crypts were measured per each section. The villi length was measured from the tip to the villi base, and then the crypt depth was measured from the base of the villi to the base of the crypt see figures 5, 6 and 7. The mean value of each replicate were obtained and used for statistical analysis.

## Statistical analysis

All the data were submitted to the ANOVA procedure for completely randomize designs using the GLM models procedure of the SAS program (SAS, 1998). A level of  $P < 0.05$  was used to denote statistical significances. Contrast comparisons (gln vs. control, glu vs. control and gln+glu vs. control) were carried out as well.

## Results

### Faecal dry matter and pH

The faecal pH and DM in different treatments for day 7, 14, 21 and 28 are illustrated in Table 2, and  $P$ -value and coefficient of variation (CV) of pH and DM are in the same table. There was no significant difference except that by day 21, faecal pH was significantly higher in chickens fed the diet supplemented with 0.5 % Glu than the chickens fed the control diet ( $P < 0.03$ ). The overall performance and carcass relative weight of broilers in different treatment groups are presented in Table 3. There were no significant differences between treatments in overall body weight.

**Table 2.** Effect of Gln and Glu on faecal pH and DM.

Treatments <sup>1</sup>	control -----	Glutamine g/kg		Glutamate g/kg		Statistics	
		5	10	5	10	CV, %	P< 0.05
<i>Feaces pH</i>							
Day 7	5.78	5.63	5.75	5.63	5.62	4.37	0.77
Day 14	5.64	5.63	5.58	5.63	5.63	1.11	0.70
Day 21 <sup>2</sup>	5.45 <sup>b</sup>	5.53 <sup>ab</sup>	5.75 <sup>ab</sup>	5.82 <sup>a</sup>	5.75 <sup>ab</sup>	3.41	0.02
Day 28	5.54	5.71	5.70	5.65	5.68	3.40	0.65
<i>Feaces DM</i>							
Day 7	22.94	23.33	22.41	22.91	23.53	4.97	0.59
Day 14	25.30	22.61	23.94	24.16	23.53	6.85	0.17
Day 21	24.28	23.90	25.04	24.45	23.66	5.90	0.60
Day 28	22.04	23.79	23.57	23.27	22.67	4.33	0.07

<sup>a-b</sup> Means within rows having same superscripts do not differ significantly.

<sup>1</sup> Means represent 5 replicate per each treatments.

<sup>2</sup> on day 21 there were significant different in faecal pH between 0.5% Glu and control group ( $P < 0.05$ ).

### Body weight development and growth performance

By day 35, there was no significant effect of supplementation of Gln or Glu at 0.5 % or 1% level on feed intake, body weight gain, FCR or slaughter yield (Table 3).

**Table 3.** Overall Performance of broiler in different treatment group (day 0- 35).

Treatments <sup>1</sup>	control	Glutamine g/kg		Glutamate g/kg		Statistics	
	-----	5	10	5	10	CV, %	P<
Body weight	15.96	16.58	16.58	16.03	14.93	10.45	0.53
Feed intake	26.84	27.87	27.64	26.70	25.85	10.42	0.79
FCR	1.68	1.70	1.64	1.66	1.74	3.84	0.17
Carcass weight /live weight	0.71	0.74	0.73	0.73	0.74	4.13	0.44

<sup>1</sup> Means represent 5 replicates per each treatment.

### Effect on *Clostridium perfringens* population in caecum

The analysis of *C. perfringens* counts in caecum is presented in Table 4. The results show that there was no significant difference.

**Table 4.** Mean *Clostridium perfringens* populations in caecum of broiler on d 34, log cfu/g

Treatments	control	Glutamine g/kg		Glutamate g/kg		Statistics	
	-----	5	10	5	10	CV, %	P<
clostridium	4.76	5.02	4.34	4.92	4.72	34.16	0.97

### Effects on carcass characteristic

The effects of dietary Gln and Glu supplementation on internal organs (liver, spleen and caecum) weights relative to the live body weight of broiler chicks and the length of the different intestinal segments (duodenum, jejunum and ileum) in different groups at day 34 are summarized in Table, 5. Statistical analysis of the data revealed that Gln and Glu supplementation had no effect on liver, spleen and caecum relative weight and length of duodenum, jejunum and ileum. However, the mean value of relative weight of caecum in group fed 0.5 % glutamine is numerically larger than the other groups, and the p-value obtained ( $P < 0.09$ ) from the relative weight of caecum indicates that there was a tendency of caecum relative weight to be statistically significant (table 5).

**Table 5.** Effect of dietary Gln and Glu supplementation on carcass traits given in relative weights and length of different small intestine segment of broiler.

Treatments	control	Glutamine g/kg		Glutamate g/kg		Statistics	
	-----	5	10	5	10	CV, %	P<
Liver relative weight	0.0196	0.0199	0.0198	0.0193	0.0203	7.98	0.88
Spleen relative weight	0.00093	0.00093	0.00089	0.0010	0.0009	19.57	0.86
Caecum relative weight	0.0036	0.0034	0.0031	0.0052	0.0033	33.21	0.09
duodenum length, cm	31.90	28.50	30.60	30.40	30.70	8.08	0.32
Jejunum length, cm	60.60	58.2	57.10	61.60	57.00	9.51	0.60
Ileum length,cm	70.60	70.20	74.10	74.20	72.20	9.16	0.80

## Intestinal morphology

The morphology of the jejunum of broilers in different treatments on day 34 is shown in Table 6 and Figure 4. Statistical analysis of data shows that Gln and Glu supplementation at the 0.5 or 1% level in the diet of broiler had no significant effect on villi length or crypt depth. Figure 4 illustrates the effects of glutamine and glutamate supplementation on jejunal villi length and crypts depth of 34 day old broiler chicken.

Table 6. Effects of Gln and Glu supplementation on villi height and crypt depth of mid small intestine of broiler.

Treatment	Control	Glutamine, g/kg		Glutamate, g/kg		Statistics	
Trait	-----	5	10	5	10	CV, %	P<
Villi length, $\mu\text{m}$	1482	1614	1713	1436	1497	14.0	0.29
Crypt deth, $\mu\text{m}$	166	190	153	150	145	20.1	0.21

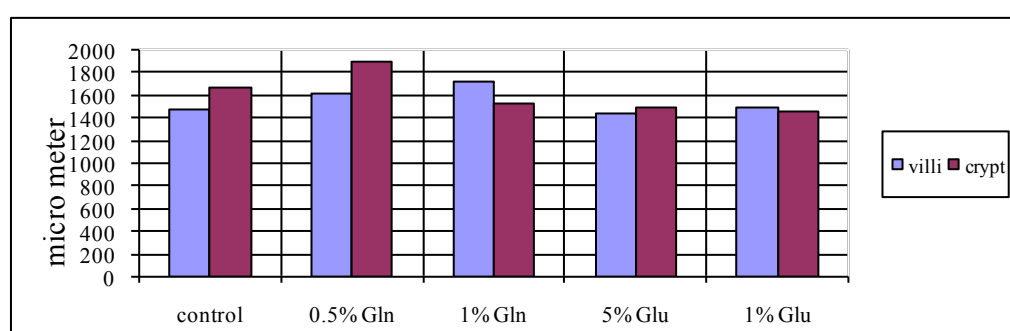


Figure 4. Effect of dietary Gln and Glu at 0.5 or 1% level on jejunum morphology of 34 d old broiler chicken. The data from crypt illustrated above were multiplied by 10.

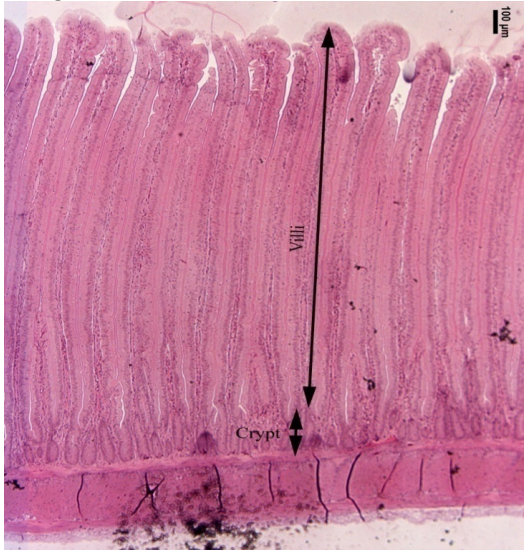
Contrast comparison of obtained data illustrated in Table 7. Regarding to villi length a contrast comparison (Gln vs. Glu) gave  $P < 0.05$ . Considering the contrast comparisons of the crypt depth there were no significant differences between treatments groups.

Table 7. Contrast comparisons of data obtained from jejunum morphology.

Villi length Contrast	P <	Crypt depth Contrast	P <
$\mu\text{m}$		$\mu\text{m}$	
Gln vs. Glu	0.05	Gln vs. Glu	0.10
Cont vs. Gln	0.14	Cont vs. Gln	0.75
Cont vs. Glu	0.89	Cont vs. Glu	0.29
Cont vs. Gln+Glu	0.45	Cont vs. Gln+Glu	0.68

Example of the intestinal photomicrography of jejunum of broiler chickens in groups fed 1-0.5% glutamine, 1-0.5% glutamate and control (without Gln and Glu) at day 34 post hatch is shown in Figures 5, 6 and 7, respectively.

1% glutamine



0.5% glutamine

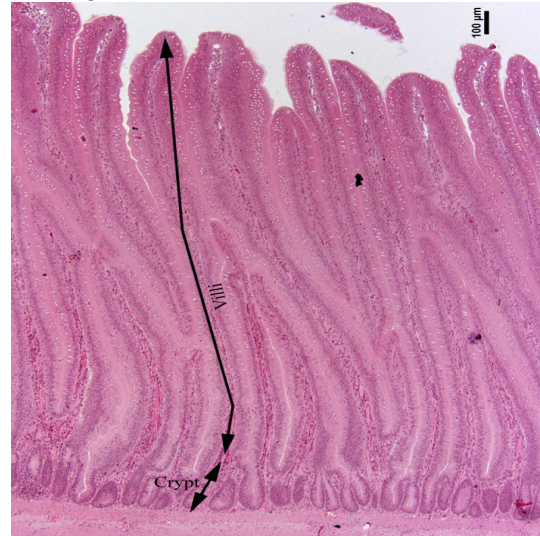


Figure 5. Photomicrography of jejunal villous and crypt of broiler chickens fed 1% and 0.5% glutamine respectively at day 34 post hatch. Bar = 100 μm.

1% glutamate



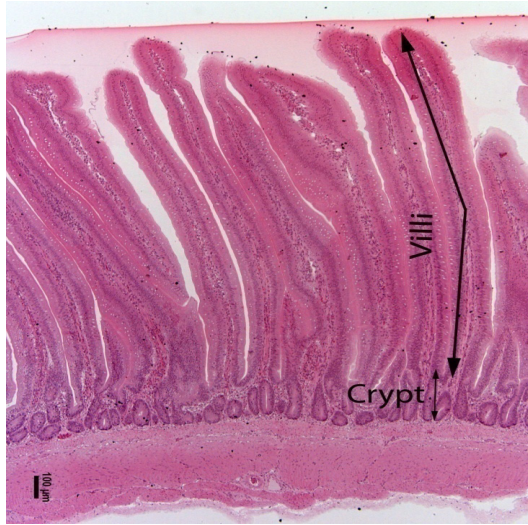
0.5% glutamate



Figure6. Photomicrography of jejunal villous and crypt of broiler chickens fed 1% and 0.5% glutamate respectively at day 34 post hatch. Bar = 100 μm.



Control (without Gln and Glu)



Control (without Gln and Glu)

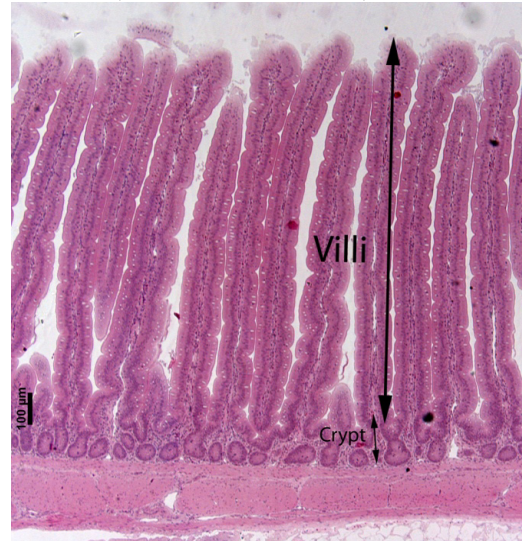


Figure 7. Photomicrography of jejunal villous and crypt of broiler chickens fed control diet (without Gln and Glu) at day 34 post hatch. Bar = 100 µm.

## Discussion

### Body weight development and growth performance

According to statistical analysis of data there were no significant effects on broilers performance due to supplementation of glutamine and glutamate at the levels 0.5-1%. Regarding broiler body weight gain the obtained data in this experiment are in agreement with (Murakami *et al.*, 2007), who observed no effect of addition of 1% Gln on whole body weight gain of broiler chickens. However, the results are not in agreement with Bartell and Batal, (2007), who observed significant increase in body weight gain of broiler chickens fed 1% Gln, but in that study it was only noted for the third week of age. Furthermore, Yi *et al.* (2005) observed that broiler fed diet supplemented with 1% Gln had highest body weight between the treatments group. In addition, Soltan (2009) observed that at week 3, 5 and 6, broiler fed diet containing 1% Gln had significantly higher body weight gain than control group and broiler fed diet supplemented with 0.5, 1.5 and 2% Gln respectively. Interestingly, Bartell and Batal, (2007) and Soltan (2009) reported that a high inclusion of dietary Gln (1.5, 2 and 4%) could be toxic and result in body weight depression. Regarding feed intake, the data obtained in this experiment is in agreement with (Murakami *et al.*, 2007; Sakamoto *et al.*, 2006) who observed no significant increase in feed intake in broilers fed diets containing vitamin E and 1% dietary Gln. As regards overall FCR the present results were in consistence with other experiments who did not report any effect of dietary Gln on broiler performance (Murakami *et al.*, 2007; Sakamoto *et al.*, 2006; Bartell and Batal, 2007).

## Effects of dietary glutamine and glutamate on carcass characteristic

Concerning spleen and liver relative weights, Glu and Gln had no significant effects on broiler spleen relative weight, which disagrees with Sakamoto *et al.* (2006); Soltan (2009); Bartell and Batal, (2007) who reported of a higher relative spleen weight when broilers were fed diets containing 1 % Gln. However, Sakamoto *et al.* (2006) reported higher spleen weights during the starter period (1-14 d), and Soltan (2009) reported higher relative spleen weights when broilers were vaccinated, which means that birds were in a challenged condition. Consistent with our findings, Soltan (2009) reported that the relative weight of the liver was unaffected by dietary Gln. Obtained data of this study indicate that dietary Glu and Gln at 0.5 -1 % levels had no significant effects on liver and spleen relative weight. Effects of dietary Glu on lymphoid organs and liver should be further evaluated for instance, when the broilers are challenged with vaccines.

## Intestinal morphology

Bartell and Batal, (2007) reported that the development of the intestinal villi in the chicken's early life could increase efficiency of nutrient utilization and enhance the growth performance. Furthermore, an increase in villi height may increase the intestinal surface area and increase nutrient absorption (Soltan, 2009; Bartell and Batal, 2007). Previous studies observed that chickens fed diet supplemented with 1% Gln had heavier intestinal weight, longer intestinal villi and deeper crypt in comparison with chickens fed glutamine free diet (Bartell and Batal, 2007; Fischer da Silva *et al.*, 2007; Murakami *et al.*, 2007; Yi *et al.*, 2005; Soltan, 2009).

Dietary supplementation of Glu or Gln at 0.5 or 1 % had no significant effects on neither villi length nor crypt depth in jejunum (Table 6). However, obtained data from contrast comparison between treatment groups (Table 7) indicated that there are significant differences between birds fed glutamine and birds fed glutamate in regard to villi length ( $p < 0.05$ , see also Fig. 4, 5 and 6), as well as there was a tendency ( $p < 0.10$ ) to higher crypt depths in birds fed Gln compared with Glu. A possible explanation for reduction in villi length in broilers fed diet supplemented with glutamate compared to the glutamine supplemented group may be due to the larger amount of the glutamic acid in diet (Table 1), which may hypothetically indicate a toxic effect of higher inclusion of glutamic acid on villi length of broiler chicken. On the other hand, we did not observe any depression in growth performance and immune related organs due to the higher inclusion of glutamic acid (Table 3 and 5) when compared with other groups. Yi *et al.* (2005) who investigated the effect of Gln on fasting and vaccinated broilers in comparison to feed groups reported that on day 2 post-hatch, there was no significant difference in villi length among treatments (feed, fast and vaccinated) but chickens in the fed group had deeper crypt than chickens in the fasting group. Furthermore, Soltan (2009) evaluated the influence of different inclusion of dietary Gln on broiler vaccinated against Newcastle disease showed that broilers fed diet containing dietary Gln had longer villi in jejunum. In addition, Fischer da Silva *et al.* (2007) observed reduction in the number of intestinal villi in chickens restricted of feed. They also observed an increase in number of intestinal villi when 1% glutamine was supplemented in to the broilers diet. The

impaired performance following a 4% Gln administration (Bartell and Batal, 2007), as earlier discussed, was accompanied with longer villi. Therefore, beneficial effects of using dietary Gln on intestinal performance might be discovered when broilers are kept in stressful condition (e.g. heat stress, vaccination or high stocking density). It may be speculated that 1% Gln could be a possible threshold for dietary Gln in diet of broiler chicken. Murakami *et al.* (2007) reported in day 14 and 41 post-hatch, broilers fed diets supplemented with 1% Gln and vitamin E had significantly longer villi in jejunum but they did not note significant differences on broiler fed only dietary Gln. Interestingly, they did not observe any improvement in broiler performance. Hence, vitamin E and Gln interaction may be a possible reason for intestinal development in that experiment.

### **Effect of glutamine and glutamate supplementation on *Clostridium perfringens* population in caecum**

*Clostridium perfringens* type A and in lesser extent type C are noticed as etiologic factors causing necrotic enteritis in broilers (Elwinger *et al.*, 1994; Drew *et al.*, 2004; Craven *et al.*, 2001; Keyburn *et al.*, 2008; Elwinger *et al.*, 1998). Necrotic enteritis is a severe and common disease in avian in every part of the world. The necrotic enteritis normally occurs in small intestine of broiler chickens during week 2 to 6 post hatch (Waldenstedt *et al.*, 1999). *C. perfringens* is frequently found in intestine of broilers. According to Elwinger *et al.* (1994), Drew *et al.* (2004), Elwinger *et al.* (1998) the number of *C. perfringens* in the intestine is normally low in healthy broiler ( $10^4$  cfu/g of digesta) but under certain condition *C. perfringens* proliferates rapidly (Elwinger *et al.*, 1998; Drew *et al.*, 2004) which may increase the incidence of necrotic enteric disease (Elwinger *et al.*, 1998).

In the present study the occurrence of the *C. perfringens* in the caecum was measured. No signs of clinical necrotic enteritis were observed during the trial. Supplementation of the diet with dietary glutamine or glutamate at the levels 0.5 or 1% had no significant effect on the number of *C. perfringens* in the caecum. The result seems acceptable, since the chickens were raised at an ideal condition and there were no signs of overgrowth of *C. perfringens*. However, there are no published studies that directly examined the effects of glutamine and glutamate on number of *C. perfringens* under adverse challenge condition.

### **Conclusions**

Birds fed glutamine supplemented diets showed higher villi length than birds fed diets supplemented with glutamate, but there were no significant differences in production performance. The current study was carried out under comparably good environmental and nutritional conditions and comparison with results from other studies indicates that some benefits, especially from glutamine supplementation, might be observed when broilers are kept in stressful conditions or when the immune system is challenged.

According to our findings, dietary glutamine and glutamate supplementation of diet had no effect on number of *Clostridium perfringens* in broiler's caecum.

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